BIOLOGY OF PLANT-MICROBE ASSOCIATIONS

Panel Manager – Dr. Bryce Falk, University of California, Davis
Program Director – Dr. Ann Lichens-Park

Research Grants in this program support studies aimed at understanding the biology of microorganisms, the interactions between microorganisms and plants, the effects of microbes on plant biology, and the influence of biotic and abiotic environmental factors on plant-microbe interactions. Studies may focus on microorganisms that have detrimental effects on plants, such as plant pathogens, or on microorganisms that have beneficial effects on plants, such as nitrogen-fixing bacteria.

2000-02672 Genes in Azotobacter Vinelandii Involved in Cellular Responses to Fixed Nitrogen

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Grant 2001-35319-10013; $200,000; 3 Years

Nitrogen fixing bacteria convert gaseous nitrogen to molecular forms needed by plants (and animals) when the supply of fixed N in their environment is low. Inhibition or repression of the nitrogen fixation process involves the products of several genes, only some of which are known and characterized. The new technology of analyzing microarrays, or DNA chips, will be applied to research using *Azotobacter vinelandii,* a free-living nitrogen fixing soil bacterium, in order to identify new genes that regulate nitrogen fixation and assimilation or are regulated by nitrogenous compounds. Approximately 15,000 random fragments of A. *vinelandii* DNA will be amplified and robotically applied to glass slides, to provide a representation of the total genome. RNA from several bacterial cultures, one supplemented with fixed N, one without fixed N, or from cultures of several known regulatory mutants, will be purified. DNA will be copied from each RNA sample in a reaction that will incorporate a different fluorescent label for each condition. The prepared microarrays will be treated with the resulting labeled cDNAs and the data overlaid, allowing the identification of fragments that hybridize to one fluorescent probe but not the other. New genes will be represented among these DNA fragments. The DNA chips will be made available to other laboratories wishing to identify new genes involved in responses to other dynamic environmental conditions. This technology can be usefully applied to analyze bacterial genomes for which a complete sequence is not yet available as is the case for a number of agriculturally-important microorganisms.

2000-02734 Virulence Mechanisms in Erwinia Chrysanthemi 3937

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Grant 2001-35319-10015; $120,000; 2 Years

Several technologies are available to explore gene expression by microorganisms grown under particular conditions. In our case, we are interested in genes expressed during the plant pathogenic phase by *Erwinia chrysanthemi*, *a* soft rot bacterium. In particular, microarray strategies and associated hardware have been developed recently that allow considerable power to be exerted to the question of gene expression. It is our assumption that genes which are upregulated during the pathogenic phase are likely to have a functional role during that phase. In the same sense, genes that are downregulated during the pathogenic phase may be deleterious during pathogenesis. We have developed technique to utilize available microarray technology to study gene expression by *E. chrysanthemi* during pathogenesis and also during growth in irrigation/river water, on the surface of plant tissues, or in a quasi saprophytic stage. These approaches should considerably advance our understanding of genes required for pathogenicity in the bacterium and permit approaches to devising disease control measures. Finally, we intend

to utilize recently devised technology to rapidly produce gene knockout strains of the bacterium for genes identified in the array work. This will permit assessment of their importance in the pathogenic phase.

2000-01256 Metabolic Profiling-Based Screen for Plant Disease Resistance Signaling Mutants

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Seed Grant; Grant 2001-35311-10200; $75,000; 2 Years

Phenylpropanoids are a class of compounds made by plants. One class of phenylpropanoid pathway derivatives is salicylic acid and its precursors. Salicylic acid is closely related to aspirin. In plants, it is essential for turning on disease resistance responses. Another class are the precursors of lignin. Lignin is a tough, water-impermeable plant compound which is involved in transport of water in plants. It also blocks penetration of plants by fungi. Plant lignin is a major limit to digestibility of forage crops by animals. Getting rid of lignin is also the major cost and major hazardous waste producing process in pulp and paper manufacturing. Regulation of salicylic acid and lignin precursor biosynthesis is complex, especially since the process is cross-regulated with that of other plant compounds important for defense against microbes and insects. A classic way in which biologists study complicated pathways is through the use of genetics. A genetic approach to this problem would involve screening for rare mutant plants which make either much less or much more of the individual chemical compounds. Through characterizing a collection of these mutants, one can hope to better understand the process. I propose to develop a method to simultaneously quantitate these compounds using a separations technology called capillary electrophoresis. A genetic screen in the model plant Arabidopsis will be based on this new method. By understanding the underlying biochemistry, progress will be made towards engineering plants for optimal disease and pest resistance and production (or non-production in crops where deleterious) of these important compounds.

2000-02746 Closterovirus Insect Interactions

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Grant 2001-35319-10098; $185,000; 3 Years

Citrus Tristeza Virus (CTV), which causes the most damaging virus diseases of citrus, has destroyed entire citrus industries throughout the world and threatens the industries throughout the US. The efficient aphid vector, the brown citrus aphid, entered Florida in 1995 and is removing the 20% of the Florida industry on the susceptible sour orange rootstock. The Texas and California industries are similarly threatened. Everyone agrees development of resistant trees is the most desirable option. However, approximately twenty years is required to produce virus-resistant trees for use in the field. The interim management procedure is to custom genetically engineer mild strains to interfere with superinfection by severe isolates. One requirement for cross protecting strains is the lack of ability to be transmitted by insects to other citrus varieties that might be susceptible to the cross protecting virus. To engineer such isolates, we first need to understanding virus insect interactions. This requires that we understand how virions are assembled and what viral gene products are needed. The objective of this project is to complete the first set of this process. We will examine the formation of virions complexes that are specifically acquired and transmitted by aphids and create mutants and hybrids to define viral genes required for aphid transmission. These studies will allow us to develop management strategies for CTV-induced diseases in a sustainable approach to managing the American citrus industry.

2000-02681 Analysis of Genes Involved in Dimorphism and Pathogeniciy in Ustilago

Maydis
Gold, S.E.
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Grant 2001-35319-10139; $196,000; 3 Years

Corn smut is a plant disease of cultivated maize. The fungus that causes this disease is a close relative of other pathogens of many grain crops. The corn smut fungus and its relatives can grow in two forms. The first form is a yeast with small dividing cells. The second form is typical of most fungi and is made up of long tubular cells or filaments. In corn smut the yeast form cannot attack the plant but the filaments can. These two forms are also common in animal and human pathogens and their form is often correlated with the ability to cause disease. We have discovered genes that control the ability of the fungus to grow in these two forms that are critical for pathogenicity. In this grant we will characterize the interactions of some of these genes with particular focus on a gene (ubc2) that encodes a novel adaptor protein. We also plan to analyze the timing of expression patterns of particular filament specific genes during the infection process to better understand the order of events. Additionally, we will attempt to identify other genes required for the maintenance of the budding growth form of the fungus. This information will have wide significance for understanding fungal growth and disease in general. It may provide new insights into novel targets for plant disease control. The results will likely have important implications for animal and human fungal diseases as well.

2000-02700 In Situ, Real-time Analysis of Virulence Gene Regulation in *Ralstonia solanacearum* During Colonization
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Grant 2001-35319-10012; $140,000; 2 Years

Ralstonia solanacearum is a widespread, economically important, soil-borne pathogen responsible for lethal wilting diseases of many important crops. Its success as a pathogen is due to at least two sophisticated regulatory networks that control production of numerous virulence and pathogenicity factors. Despite intense study, little is known about how *R. solanacearum*'s regulatory systems function in planta, because most of the work was performed using bacteria in artificial culture. Fortunately, engineering *R. solanacearum* to make green fluorescent protein (GFP) is revolutionizing our ability to track its movement on and in host plants and to monitor changes in gene expression in situ and in real time. To realize the full potential of GFP, we first will develop a new transposon-based system that can be used to create special strains of the pathogen (and most other Gram-negative bacteria) that fluoresce only when the desired virulence or pathogenicity gene is expressed. These strains will be used to investigate the development of bacterial colonies on artificial and tomato root surfaces and the concomitant gene regulation. We will be especially interested to see how gene expression is coordinated with attachment and twitching motility. This application of GFP to study *R. solanacearum* in situ will reveal totally unexplored aspects of this pathogen's behavior, will provide new insight into fundamental aspects of microbial growth on plants, and will contribute significantly to the little that is known about gene expression in sessile bacteria.

2000-01192 Equipment for Agricultural and Environmental Biotechnology Research
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University of Hawaii, Manoa; Department of Molecular Biosciences and Biosystems Engineering; Honolulu, HI 96822
Equipment Grant; Grant 2001-35311-10195; $12,000; 1 Year

This is an equipment grant for purchasing a refrigerated Centrifuge for use in several research projects by a group of researchers engaged in agricultural and environmental biotechnology research at the University of Hawaii. Some major objectives of these projects are

to: (a) determine the role of certain compounds in the plant root exudates in inducing bacterial genes in the rhizosphere (b) characterize a novel alternate sigma factor in *Rhizobium* (c) clone genes for mimosine biosynthesis from *Leucaena*, (d) characterize genes for exopolysaccharide synthesis *Rhizobium*, (e) improve chicken skeletal muscle growth by immuno-modulation of myostatin bioreactivity (f) develop strategies for enhancing heterologous protein production in perfusion plant cell cultures, (g) synthesize pyrones in the tissue culture of kava, (h) develop genetically engineered antibodies for monitoring polynuclear aromatic hydrocarbons, (i) determine the fate of pollutants in the environment, and (j) determine the effects of certain pollutants on humans and endangered terrestrial and marine organisms. The requested centrifuge will remove a major disadvantage for the researchers and will become a part of the facility and the infrastructure of the new Agricultural Sciences building.

2000-02736 Regulation of Barley Yellow Dwarf Virus Gene Expression
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Grant 2001-35319-10011; $200,000; 3 Years

Virus genomes are small and densely packed with genes and regulatory signals. This research investigates gene expression of the serious plant pathogen, barley yellow dwarf virus (BYDV). BYDV encodes six genes on one genomic RNA. As the RNA is replicated, less-than-full-length subgenomic RNAs are generated. These serve as mRNAs for internal (on genomic RNA) viral genes. The first aim is to test our hypothesis that different BYDV subgenomic RNAs may arise by different mechanisms. We will examine the effects of designed mutations on subgenomic RNA synthesis in BYDV-infected oat cells. We will also test our hypothesis that one of the subgenomic RNAs facilitates a switch from genomic RNA translation (protein synthesis) to subgenomic RNA translation by competition. The second aim is to determine the timing of viral RNA and protein accumulation in infected cells, and to test our hypothesis that viral RNAs may shut off host translation by the competitive mechanism. The green fluorescent protein gene has been introduced into the viral genome for detection of infected cells. These will be separated from uninfected cells by flow cytometry, and host protein synthesis will be compared between the two pools of cells. To study the effects of the BYDV translational regulatory sequences independent of other BYDV sequences, we will express isolated BYDV RNA sequences from a brome mosaic virus vector and possibly in transgenic plants. This research will improve our understanding of replication and gene expression relevant to many plant and animal RNA viruses, and of translation processes in general

2000-01132 Coevolution of *Melampsora* Rust Pathogens of *Populus*
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Equipment Grant; Grant 2001-35311-10159; $23,333; 1 Year

The overall project goal is to gain insight into the evolution of pathotype-specific or general rust resistance in plants. *Melampsora* rust fungi occur on all species of *Populus* (cottonwoods, or poplars), and new research is revealing narrower host specificity in these relationships than previously believed. Within a well-defined *Populus – Melampsora* pairing, pathotype specificity may also be present, as in the *Populus deltoides – Melampsora medusae* (rust of Eastern cottonwood) system, or absent, as in the *Populus trichocarpa – Melampsora occidentalis* (rust of Western black cottonwood). The presence of pathotype-specific resistance greatly complicates breeding for resistance; new pathotypes or races of rust may defeat resistant cultivars, as has happened so often with cereals and other agricultural crops. The applied

significance of pathotype specificity to agriculture and forestry justifies an attempt to understand its evolution by ultimately mapping the character on a phylogeny of *Populus*. Supporting objectives include the initial description of the remaining undescribed species of *Melampsora* that occur on *Populus*. Descriptions would include determinations of host range in the requested equipment (i.e., Conviron BDR16 plant growth chamber). At the same time, determinations of the presence or absence of pathotype-specific resistance in each *Populus – Melampsora* pairing can be made using the same chamber. The BDR16 would also be used by the PI in new research, both pending and projected, within a new Forest Pathology Project at the University of Idaho.

2000-02818 Characterizing the *PMK1* Pathway Regulating Fungal Pathogenesis in *Magnaporthe grisea*

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Grant 2001-35319-09924; $300,000; 3 Years

All fungal pathogens have to recognize host plant surfaces and penetrate into the tissues for successful infection. The fungus, *Magnaporthe grisea,* causes blast disease of economically important crops such as barley, wheat and rice, and it has been developed into a model system for studying fungal infection processes. Recently, a gene referred to as *PMKI* for Pathogenicity MAP kinase has been implicated in regulating infection structure formation and infectious growth by the blast fungus. Studies with several fungi have indicated that the *PMKI* gene is present in many fungi and is required for plant infection. However, knowledge of the exact function of this gene in plant pathogenic fungi is very limited. The major objective of this research is to further determine the functions of the *PMKI* gene and the pathway that signals production of infection structures. These experiments will identify the signal inputs and outputs of this important pathway. Another objective is to identify and characterize other genes that are regulated by the *PMKI* gene. Some of these genes may be novel pathogenicity factors in fungal pathogens. The third objective is to examine the expression and activation of the *PMKI* gene during infection structure formation. This research will clarify the relationship between surface recognition signaling and gene expression during plant infection. Overall, results from this research will help us better understand the mechanisms that regulate plant infection in *M. grisea* and other fungal pathogens. Ultimately, this study will provide important information for developing more effective disease control strategies.

2000-02698 Characterization of the Pathogenicity Protein AvrXa7

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Grant 2001-35319-09851; $300,000; 3 Years

The product of the *avrXa7* gene (AvrXa7) is secreted by the bacterium *Xanthomonas oryzae* pv. *oryzae* into the cells of the host plant rice during infection and is required for pathogenicity. Parallels with animal pathogen models suggest that the bacteria may use the protein to interfere with signaling pathways and biochemical reactions associated with general defense responses of the host. How AvrXa7 and related proteins function in virulence is unknown. This proposal presents the hypothesis that the product of the avirulence gene *avrXa7* is produced by the bacterium and enters the host cell nucleus. Evidence is presented both from published and preliminary data suggesting that AvrXa7 has features in common with eucaryotic transcription factors (gene regulatory proteins). We will determine the capacity of AvrXa7 to bind DNA and direct the expression of host genes. The specific objectives are: (1) Determine if AvrXa7 can bind DNA and characterize the regions of DNA binding and (2) characterize nature of the bound DNA and determine if AvrXa7 can promote gene expression in plants. Insight into the strategies of pathogens may be gained if the targets of the avirulence proteins, acting in their

virulence capacity, can be identified. Knowledge of the critical features of virulence determinants and their targets will undoubtedly lead to new genetically deployed strategies for crop protection. From a broader perspective, *X. oryzae* pv. *oryzae* is the most economically and most important bacterial pathogen of rice. The system is one of the few bacterial\monocot systems studied in detail.

2000-02733 Nonhost Resistance Mechanisms in Arabidopsis
Zhou, J.
Kansas State University; Manhattan, KS 66506
Grant 2001-35319-09847; $140,000; 1 Year

Any given plant species is resistant to a vast majority of phytopathogens. This resistance, called nonhost resistance, holds great potential in controlling phytopathogens in a durable and nonspecific fashion. However, genetic mechanisms controlling nonhost resistance in plants are largely unknown. The proposed research will develop a new approach to identify major genes in *Arabidopsis thaliana* that control nonhost disease resistance. The identification and isolation of plant genes conferring nonhost resistance should provide novel means for improving disease resistance in crop plants.

2000-02805 Isolation of Maize Genes Controlling Defense Reactions to Pathogens of Cereals
Hulbert, S.H.; Leach, J.E.; Trick, H.
Kansas State University; Department of Plant Pathology; Manhattan KS, 66506-5502
Grant 2001-35319-10014; $130,000; 2 Years

Disease resistance genes in plants code for proteins that recognize the presence of pathogens and then induce defense responses. Transfer of disease resistance genes to crop species from close relatives is a common breeding tool. It is limited, however, by the lack of donor species that can be hybridized with any given crop species, and by the extent of genetic variation in these species that has not already been utilized. A future trend may be to transfer genes from much more distantly related species by recombinant DNA techniques. Over twenty plant disease resistance genes have now been characterized at the molecular level, so we now know what they look like. As a result, resistance genes have become easier to isolate. Experimental transfers of resistance genes between close relatives in the tomato family have indicated some resistance genes will function after transfer to a different species, as long as that species is related. Our long-term objectives are to determine whether resistance genes will function after transfer between cereal species. We have genetically characterized two genes in maize that we will use for this purpose. The *Rxo* gene confers a defense reaction to the rice bacterial streak pathogen, *Xanthomonas oryzae* pv. *oryzicola*. The *Rpa* gene confers a hypersensitive reaction to the sorghum bacterial stripe pathogen, *Pseudomonas andropogonis*. We have candidate DNA sequences (gene fragments) that code for these genes. We will isolate these candidate genes from maize and verify that they code for these resistances by reintroduction into maize. If we can verify that these sequences code for the resistance genes, we will begin to engineer them into rice and sorghum to determine if they can control these diseases.

2000-02849 Roles of Pathogen-Inducible EREBP-Like Sequences in Disease Resistance
Warren, R.F.
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Postdoctoral Fellowship; Grant 2001-35319-09902; $90,000; 2 Years

The development of novel strategies to prevent plant disease requires knowledge of specific molecular mechanisms that plants utilize to confer pathogen resistance. Within plants, there exists a unique class of genes termed ethylene-responsive element binding proteins, or EREBPs, which in response to environmental cues function to turn on or turn off other genes.

Using the model plant *Arabidopsis thaliana*, we have identified a subset of EREBPs that may be involved in regulating pathogen defense responses within the plant. Potentially, the manipulation of EREBPs could activate defense responses in plants to confer disease protection. In order to understand more precisely the roles that EREBPs may play in protecting a plant from disease, it is necessary to place them in the context of pathogen resistance signaling networks within the plant. This is most easily accomplished by conducting experiments in previously characterized mutant plants that are affected in molecular pathways involving pathogen resistance. By comparing the expression of EREBPs in normal and mutant plants, we can determine their potential position in a disease resistance pathway. We will also examine the effect of compounds that enhance disease resistance on the expression of EREBPs. Additionally, by infecting plants with multiple pathogen strains, we will determine if specific EREBPs are involved only in response to specific strains or if a broad range of pathogen strains affects their expression. We plan to establish a direct role of EREBPs in pathogen resistance by knocking out or overexpressing specific EREBP genes and examining the effect on pathogen resistance.

2000-02684 Molecular Basis of Disease in a Virus-Infected Plant Pathogenic Fungus
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Grant 2001-35319-10010; $190,000; 3 Years

Virus-induced diseases and hypovirulence in plant pathogenic fungi provide excellent opportunities for fundamental studies aimed at developing novel biological control measures. In this regard, the dsRNA totivirus *(Helminthosporiun~ victoriae l90Sv~rus;* Hv190SV) and associated chrysovirus-like Hv145SV infecting the Victoria blight fungus *Helminthosporium (Cochliobolus) victoriae* are of special interest because they are associated with a disease of their fungal host. The overall goal of the project is to explain the molecular basis of disease in H. *victoriae* with emphasis on a recently discovered multifunctional cellular oxidase (Hv-p68) with RNA-binding and phosphorylating activities that accumulates in diseased fungal isolates, and on a broadspectrum antifungal polypeptide "victoriocin" secreted by virus-infected isolates. The objectives are: to elucidate the genome organization and expression of the Hv145SV, to transform virus-free H. *victoriae* with full length cDNA clones of the two viruses, to disrupt the lIv-p68 gene in virus-infected isolates, and to isolate the gene encoding the antifungal polypeptide, victoriocin. At the present time, control of plant pathogenic fungi is a formidable task due to the lack of appropriate disease control strategies. In addition to the health hazards and the risks to the environment, the use of fungicides is often costprohibitive. The need for novel biological control measures to combat fungal diseases cannot be overstated. The proposed research seeks to pursue two novel approaches: activation of a multifunctional cellular protein leading to a diseased phenotype, and transgenic resistance to fungal pathogens through plant transformation with a gene encoding a broad-spectrum antifungal polypeptide.

2000-02661 Modeling the Resistance/Susceptibility of *Arabidopsis thaliana to Erysiphe orontii*
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Postdoctoral Fellowship; Grant 2001-35319-09850; $89,990; 2 Years

The resistance or susceptibility of a host plant to a given pathogen is determined by a complex interplay of plant and pathogen factors. The use of *Arabidopsis thaliana* as a model plant has led to the isolation of *Arabidopsis* mutants with increased susceptibility or resistance to a variety of pathogens and to the elucidation of plant defense pathways. Many of these defense pathways are activated even in susceptible plants, and it appears that the timing, magnitude, and/or extent of these plant defenses may determine the success of a pathogen infection. The long-term objective of my research, which this proposal initiates, is to model the

resistance/susceptibility of *Arabidopsis* to *Erysiphe orontii,* a powdery mildew, with the goal of elucidating the critical pathways, key regulatory factors, and specific defense compounds resulting in *Arabidopsis* resistance/susceptibility. To isolate and characterize critical components of the *Arabidopsis* defense response, mRNA expression profiling and analytical quantitation of antimicrobial compounds will be performed with *Arabidopsis* mutants that have altered susceptibility to *E. orontii.* Genes that are coordinately regulated will be analyzed to discover shared regulatory elements and to begin to place individual genes in a functional framework. As critical components of the *Arabidopsis* defense against *E. orontii* are defined, specific equations detailing these components will be derived and integrated into a formal model. The resultant data and initial model will facilitate the identification and understanding of key pathways, regulatory factors, and compounds involved in plant defense against powdery mildews. This should result in novel targets for the control of these common agronomic pathogens.

2000-02854 17th North American Conference on Symbiotic Nitrogen Fixation
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University of Minnesota; Department of Soil, Water, and Climate; St. Paul, MN 55108
Conference Grant; Grant 2001-35319-09900; $10,000; 1 Year

Since its inception, more than 33 years ago, The North American Conference on Symbiotic Nitrogen Fixation (formerly the *Rhizabium* Conference), on an approximately two year cycle, has been a major vehicle by which scientists studying the symbiotic interaction of nitrogen-fixing bacteria and leguminous plants have exchanged current information. This year's conference will be held at Laval University, Quebec City, Canada from 23-28 July. The subject matter of the conference is diverse and ranges from the very basic to the applied. Selected topics for this years meeting includes; genomics, plant and microbial physiology, ecology, metabolism, differentiation and regulation in plants and bacteria, plant-microbe signaling, taxonomy and evolution, agricultural and environmental applications of N2-fixing microorganisms, and legume inoculation technology. The scientific program will consist of 12 plenary sessions and continuous poster sessions. The informal atmosphere of the conference is conducive to small group discussions. Moreover, the size, format, and low registration fee of the conference encourages participation by graduate students and postdoctoral associates. The proceedings of the 17th North American Conference on Symbiotic Nitrogen Fixation will be most likely published by Kluwer Academic Publishers and selected manuscripts will be published as a special addition in the Canadian Journal of Microbiology.

2000-02679 *Characterizing Genes Regulating Octadecanoid and Salicylic Acid Signaling*
Bergey, D.R.
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New Investigator Award; Grant 2001-35319-09843; $100,000; 2 Years

Systemin, jasmonic acid (JA), and salicylic acid (SA) mediate plant defense responses to pests and pathogens, and the signaling pathways governed by these potent gene regulators overlap. We will use high-resolution serial analysis of gene expression (SAGE) to identify and characterize genes involved in mediating cross talk among these defense gene activators.

Physical damage by pests or wounding initiates the processing and rapid mobilization of a polypeptide signal, systemin, throughout plant tissues. Upon interaction with target cells, systemin activates a biochemical cascade (the octadecanoid pathway) that results in the biosynthesis of JA and regulation of defense-related genes encoding systemic wound responsive proteins (SWRP's). Transgenic tomato plants overexpressing prosystemin appear normal, but constitutively express SWRP genes in the absence of wounding. The transgenic plants thus provide a unique experimental system for investigating and characterizing a constitutively activated plant defense signaling circuit.

Salicylic acid (SA) plays a fundamental role in many pathogen-mediated signaling

pathways, and activates a distinct group of genes encoding pathogenesis-related (PR) proteins. Application of SA to plants before wounding blocks systemin-mediated SWRP gene expression, and in general SA- and systemin-mediated plant defense pathways are mutually antagonistic. Other lines of evidence also confirm that cross talk between these major defense pathways occurs, yet genes governing the interaction between these vital signaling circuits have not been identified.

Characterization of genes that control SA- and systemin-mediated defense pathways will provide insight into the regulatory interplay that coordinates plant defense responses to a diverse range of biological, physical, and environmental stresses.

2000-02682 New Roles for Mannitol and Mannitol Dehydrogenase in Plant-Pathogenactions

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Grant 2001-35319-09849; $210,000; 3 Years

The sugar alcohol mannitol and its catabolic enzyme mannitol dehydrogenase (MTD) play established roles in photosynthesis as well as in salt and oxidative stress tolerance. Recent research also implicates mannitol and MTD in plant-pathogen defense. Plants use reactive oxygen species (ROS) both as antimicrobial agents and as signal molecules to initiate diverse defense responses. Successful pathogens appear to have evolved myriad ways to evade these defenses. For instance, many plant pathogens make mannitol, a potent quencher of ROS, and mannitol production is necessary for pathogenicity by such diverse fungi as the tomato pathogen *Cladosporium* and the human pathogen *Cryptococcus*. If fungal pathogens secrete mannitol to quench ROS produced by and mediating host defenses, then plants that can degrade pathogen-produced mannitol should be more resistant to pathogen attack. In fact, our work suggests that pathogen-induced expression of the mannitol catabolic enzyme MTD may be a fairly general response to pathogen attack. To date, in addition to finding MTD in mannitol-containing plants (e.g. celery, parsley and snapdragon), three non-mannitol plants (tobacco, tomato and *Arabidopsis)* have been found to contain pathogen-induced MTD. Furthermore, constitutive ectopic expression of celery MTD in tobacco confers significantly enhanced resistance to the mannitol secreting leaf spot fungus *Altemaria.* In short, mannitol dehydrogenase appears to represent a new class of pathogen resistance gene, with exciting potential for introducing increased fungal resistance in plants. In this proposal we present experiments designed to investigate the mechanisms of resistance and define the contribution of this gene to pathogen resistance in plants.

2000-05979 Secretion Properties of the Type 111 Secretion System of *Pseudomonas syringae*

Alfano, James R.
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Grant 2001-35319-10019; $240,000; 3 Years

In general, the experiments described in this proposal are designed to reveal secretion properties of the type 111 (Hrp) secretion pathway of *Pseudomonas syringae*. One major goal is to identify and delimit the secretion signals utilized by one type III-secreted protein, HopPsyA (HrmA). Based on preliminary evidence, HopPsyA appears to use a molecular chaperone and we will also test whether the *hopPsyA* mRNA has its own secretion signal. Other experiments will exploit the recent progress that researchers have made in detecting type III-secreted proteins in culture supernatants to isolate additional proteins that travel the Hrp pathway. To properly categorize the type III-secreted proteins, we are developing a series of assays designed to identify effector proteins that are translocated into eucaryotic cells. As an ongoing effort to

identify effector targets in the plant, our last objective uses the yeast 2-hybrid system to screen for plant proteins that interact with a protein, HopPtoB, that we have recently identified as a type III-secreted protein. The research described in this proposal should provide important information about type III secretion systems and be of broad interest to researchers studying bacterial pathogenesis. Increasing our understanding how type III pathways deliver virulence proteins to the interior of host cells may help in the design of pharmaceuticals and/or agricultural pesticides or herbicides.

2000-02817 Bacterial Pathogenesis of Fungal Plant Pathogens

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Grant 2001-35319-02817; $180,000; 3 Years

Biological controls are potential alternatives to pesticides for plant disease control. However, acceptance of biocontrols has been slowed by inconsistencies in their performance. *Stenotrophomonas maltophilia* strain C3 is a bacterium capable of controlling several plant diseases. Strain C3 is also capable of colonizing and lysing fungal mycelia, and is considered a pathogen of fungi. This pathogenic interaction is thought to be the major mechanism involved in biocontrol activity. Traits expressed by strain C3 contributing to the pathogenic interaction, however, remain unclear. In this proposal, we wish to genetically identify traits involved in strain C3 pathogenesis of fungi, and evaluate the role of these traits in biocontrol. Strain C3 produces extracellular enzymes, including chitinases, which are capable of degrading fungal cell walls. Also, strain C3 is thought to possess a type III secretion pathway, which is a central mechanism for bacterial pathogenesis of animals and plants. Genes encoding chitinases and a putative type III secretion pathway will be identified, molecularly characterized and targeted for mutagenesis in strain C3. Mutants will be evaluated for biocontrol activity against damping off of sugar beet, Bipolaris leaf spot of tall fescue and summer patch disease of Kentucky bluegrass. Mutants will also be evaluated for pathogenic interactions with fungi. These studies are expected to provide a better understanding of bacterial traits that contribute to pathogenesis of fungi, and establish the roles of these traits in biocontrol activity. By understanding of the biocontrol mechanism(s) expressed by strain C3, improved biocontrol efficacy and performance consistency are expected.

2000-02644 Evaluation of Cause and Acquisition of Virulence in *Pyrenophora tritici-repentis*

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Grant 2001-35319-10017; $190,000; 3 Years

Tan spot of wheat is a destructive disease throughout major wheat growing areas of the world. Yield losses have been reported up to 50% in the central plains of the US and Canada. Increases in disease incidence have been attributed to changes in cultural practices including shifts from conventional tillage to conservation- and zero-tillage practices, shorter rotations and continuous wheat cultivation, the growth of highly susceptible cultivars, and the change from stubble burning to its retention.

The goal of our research program is a description at the molecular level of the events that determine disease development in tan spot of wheat caused by the fungus *Pyrenophora tritici-repentis.* Our goals include identification and characterization of genes involved in disease development and host specificity, and mechanisms of susceptibility and resistance. Fungal plant pathogens that produce host-selective toxins (HSTs) are ideal organisms to address these objectives because HSTs are considered to be causal in disease development. The work outlined in this proposal will evaluate and characterize a potential pathogenicity factor, develop an

understanding of the genetic relationship between pathogenic and non-pathogenic isolates of the fungus, and evaluate the structural requirements of the major toxin (ToxA) to gain insight into how this toxin exerts its influence in sensitive wheat cells. Understanding the molecular genetics of toxin production will provide insight into mechanisms of disease development. In turn, this information could be exploited for control of this wide-ranging and serious disease on wheat.

2000-02677 Spatiotemporal Dynamics of Disease in Homogeneous and Heterogeneous Crops

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For practical reasons, most field research in plant pathology is done in small experimental plots. However, epidemics can often expand over much larger areas in commercial agriculture. Theory has been developed to suggest that epidemics expand as travelling waves of constant speed. However, there also are both theoretical approaches and empirical data which suggest that epidemic speed may increase in both time and space for pathogens that are wind-dispersed. Resolution of this issue has critical implications for evaluation of disease control practices: increasing epidemic speed over time and space implies that disease management practices become relatively more effective when used in commercial production as compared to small-scale experimental field plots.

Experiments will be conducted to study the spread of wheat stripe rust in large field plots at two locations and over three seasons. Epidemic spread from initial foci of disease will be monitored in time and space. An emphasis will be to compare epidemic spread in plots of a single wheat variety versus a mixture of two varieties. Observations suggest that variety mixtures have greater impacts on reducing the severity of epidemics at larger spatial scales' but actual data are lacking.

Data will be used to test three hypotheses that are predicted by the theory that epidemic velocity increases as disease expands in time and space. Testing these hypotheses will better enable us to evaluate disease management options in commercial-scale agriculture and to better predict the value of crop genetic diversity for reducing epidemics.

2000-02646 Viral and Plant Suppressors of Gene Silencing

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Grant 2001-35319-09848; $300,000; 3 Years

Post-transcriptional gene silencing (PTGS) is a fundamental regulatory mechanism operating in plants, animals and fungi. PTGS serves as an antiviral defense mechanisms in plants and may play a similar role in other organisms. We have identified a plant virus protein, the helper component-proteinase (HC-Pro) of potyviruses, that suppresses PTGS and thereby acts as a counter-defense. In preliminary results, we have identified a cellular protein that interacts with HC-Pro in the yeast two-hybrid system. This cellular protein is a novel calmodulin-related protein (termed rgs-CaM) which, like HC-Pro itself, suppresses PTGS. In preliminary results we show that rgs-CaM expression is induced in plants where PTGS has been lifted by HC-Pro. Here we propose to use rgs-CaM as a tool to dissect the mechanism of silencing. The research has three specific aims. The first aim is to use rgs-CaM in protein interaction assays to look for other plant proteins involved in PTGS. The second and third aims are designed to determine if four other recently discovered viral suppressors of PTGS also mediate suppression of silencing via interaction with rgs-CaM or induction of rgs-CaM gene expression. The investigation of suppressors of PTGS is useful in a practical sense. These proteins have significant potential to improve yield in technologies that use plants to express foreign gene products. Given the antiviral nature of gene silencing in plants and the indications

that PTGS is an ancient mechanism in eukaryotic organisms, the work could lead to development of antiviral strategies in both plants and animals.

2000-02656 Genetic and Functional Analysis ofthe Syringomycin/Syringopeptin Synthetase System

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The bacterium, *Pseudomonas syrtngae pv. syringae,* causes serious diseases of many cultivated plant species in the USA. The pathogen produces two phytotoxins, called syringomycin and syringopeptin, which have important roles in disease development. The goal ofthis research is to understand how the pathogen invades susceptible plants concenh~a~g on the important role oftoxins in the disease process. Nearly 2% of the pathogen's DNA is dedicated to the synthesis ofthe two toxins, end this research is focused on characterizing this relatively large region ofthe bacterial genome that is critical to being a pathogen.

The objectives are to genetically and functionally define how the two toxins are biosynthesized, and characterize important regulatory genes that control toxin production during disease development. We will fully sequence the 135-kb DNA region encoding the toxin clusters, overexpress biosynthesis genes or modules for enzymatic studies to define their specific function, and evaluate the contribution of each gene to bacterial virulence in cherry fruits. Modem molecular genetic tools, such as reporter gene fusions, will be used to analyze gene express~on under various environmental conditions. Regulatory genes clustered at the lefc and right border regions ofthe toxin clusters will be characterized, end their role in controlling toxin production will be analyzed. These studies will generate new concepts in plant-pathogen interactions that are crucial to developing new disease control practices.

2000-02809 Ecological Genetics of the Cheatgrass - Head Smut Pathosystem

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Cheatgrass *(Bromus tectorum) is* an exotic winter annual grass that dominates millions of hectares of degraded rangeland in the western United States. Head smut *(Ustilago bullata) is* a systemic seedling-infecting fungal pathogen that commonly infects cheatgrass populations. The research objective is to investigate the ecological genetics ofthis pathosystem as a necessary prelude to manipulation of the pathosystem for biocontrol of cheatgrass. Polymorphism for resistance in the host and for pathogenicity in the pathogen will be evaluated in greenhouse inoculation trials using host inbred lines and head smut isolates from eight cheatgrass populations. The geneti4s of pathogenicity will be examined, and molecular markers linked to virulence genes will be developed. Among-year variation in frequency of resistance phenotypes in the host and pathogen races in the pathogen will be investigated in the field for four cheatgrass populations occupying contrasting habitats and varying in population size and degree of isolation. Microsatellite markers will be used as a tool for population genetic analysis of both host and pathogen populations. Information generated from field studies will be used to test altemative ecological genetic models of host-pathogen coevolution. The pathosystem associated with a large, contiguous cheatgrass population is expected to conform more closely to a frequency-dependent selection model, while those associated with smaller, more fragmented populations are expected to conform more closely to a metapopulation model. In addition, the hypothesis that head smut populations possess ecotypic variation in response to environmental factors will be tested in controlled-environment experiments.

2000-02798 Ammonia Switch-off in Methanococcus

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Grant 2001-35319-09927; $270,000; 3 Years

Biological nitrogen fixation, carried out by a variety of bacteria and archaebacteria (Archaea), is an important source of nitrogen for crop plants. Organisms regulate their nitrogen-fixing activities in order to adjust to the nitrogen conditions and other conditions of their habitat. The molecular mechanism of this regulation varies with different taxonomic or phylogenetic groups of organisms. Therefore, it is not surprising to find that the Archaea, which belong to a group that diverged from other organisms at an extremely ancient time, reveal important variations on paradigms of regulation. In this project, we will investigate aspects of nitrogen-regulatory mechanisms in one species of Archaea, *Methanococcus maripaludis*. We will focus on a mechanism that rapidly inactivates nitrogen fixation when the organism encounters already-fixed nitrogen, called ammonia switch-off. We will determine what sort of modification of the enzymes for nitrogen fixation occurs, and what genes are required for the switch-off process. The results should enhance our perspective on nitrogen-regulatory mechanisms in general as well enhance our understanding of the mechanism in this particular species.

2000-02690 Genetics of Symbiotic Effectiveness in Legumes

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Grant 2001-35319-09923; $210,000; 3 Years

Legumes such as alfalfa, peas and soybean can obtain the nitrogen they need by participating in a symbiotic association with bacteria that can transform atmospheric nitrogen into a biologically useful form. How well this symbiotic nitrogen fixation contributes to the growth of the legume is called the effectiveness of the association. Effectiveness depends significantly on interactions between the symbionts. The plant contributes to these interactions by recognizing appropriate bacteria, developing a root nodule in response to bacterial signals and supporting the metabolism needed for bacterial nitrogen fixation.

The number and location of plant genes involved in determining effectiveness is unknown. Breeding more effective crop plants has been hampered by the complexity of using nitrogen fixation as a quantitative trait. Two developments could change this situation: a better assay for nitrogen fixation and improved methods of genetic analysis. We have taken the first steps toward developing a more rapid and more reproducible way to measure nitrogen fixation using a new mass spectroscopy method and will push this method further to allow it to be used to evaluate effectiveness. We will use the method to analyze the heritability of symbiotic effectiveness using recombinant inbred lines of pea and *Medicago trmcatula,* a relative of alfalfa, in order to associate the gene(s) responsible for phenotypic variation in effectiveness with quantitative trait loci. Success in this effort will help identify plant genes responsible for effectiveness and will contribute to breeding strategies that maximize effectiveness in agronomically useful cultivars.

2000-02675 Phyto-Management of Microbial Communities to Enhance Tree Growth in Replant Soils

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Establishment of an orchard on a site previously planted to apple often results in poor growth and death of new apple trees, a phenomenon termed apple replant disease. The disease is commonly controlled through the application of pre-plant soil fumigants, including methyl

bromide. The impeding ban on use of methyl bromide and potential regulatory restrictions on other broad-spectrum fumigants place in doubt the long-term availability of suitable chemical measures for control of replant disease. All soil ecosystems possess microorganisms with the ability to control plant diseases. Enhancing populations and activity of these organisms has the potential to serve as an environmentally sensitive and biologically sustainable means to control soilborne plant pathogens. Preliminary studies demonstrated that cultivating replant soils with wheat selects for a population of microorganisms that can suppress the fungal pathogens that cause apple replant disease. The event occurred in a wheat cultivar-specific manner and was not induced by other grasses. Disease suppression appears to be due, in part, to selection of specific bacteria belonging to the fluorescent *Pseudomonas* spp. Our goal is to identify the fluorescent *Pseudomonas* genotypes that contribute to suppression of apple replant disease and determine the plant traits that are key in the selection of these suppressive bacteria. This information will be significant for identifying wheat cultivars with a superior ability to select for microbial communities that enhance apple growth on replant sites. These studies may also provide the framework for selection of apple rootstocks that support pathogen-suppressive fluorescent pseudomonad genotypes.

2000-02642 Role of Polyunsaturated Fatty Acids in the *Aspergillus*/Seed Interaction
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Grant 2001-35319-10996; $210,000; 3 Years

Grain and legume seed are attacked by relatively few fungal genera. However the fungi that do colonize seed, most commonly members of the genera *Aspergillus*, *Fusarium* and *Penicillium,* cause tremendous yield loss through tissue destruction as well as a significant health problem by the production of mycotoxins in the seed. The mycotoxigenic fungi are facultative pathogens that form intimate associations with grain and legume seeds. Under certain environmental conditions, the fungi will produce copious amounts of toxic, teratogenic and carcinogenic toxins in these living seeds. At other times the fungi will colonize the seed but not produce mycotoxins. These diseases, considered one of the most serious and challenging agricultural problems, result in the most yield loss of any plant disease type, well into billion dollar losses every year. Aside from the yield loss, there is also the hard to measure health loss in animals and humans that eat contaminated food.

We provide evidence that there is a sophisticated lipid mediated interaction between *Aspergillus* spp. and their host seed. We show that seed polyunsaturated fatty acids affect the ability of *Aspergillus* to produce infection structures (e.g. spores) and to produce the carcinogenic mycotoxin called aflatoxin. Moreover, *Aspergillus* infection of seed regulates the expression of seed lipid metabolism genes, lipoxygenases, that produce some of these polyunsaturated fatty acids. The overall goal of this proposal is to test the hypothesis that linoleic acid (a polyunsaturated fatty acid) and linoleic acid derivatives from seed are virulence factors for successful colonization of seed by *Aspergillus* spp. and possibly other seed attacking fungi. Confirmation of this hypothesis will lead to strategies to decrease colonization and subsequent aflatoxin contamination of seed by the aspergilli.

2000-02669 Genetic Suppressors of *Arabidopsis dnd* Disease Resistance Mutants
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Grant 2001-35319-09888; $180,000; 3 years

Genetically controlled plant disease resistance has direct utility in the control of agricultural diseases. *Arabidopsis dnd1* and *dnd2* mutants exhibit gene-for-gene disease resistance with little or no HR cell death, and also exhibit elevated broad-spectrum resistance. We recently discovered that *DND1* encodes a cyclic nucleotide-gated ion channel that impacts

plant disease resistance; the mutated *dnd1-1* gene carries a stop codon that would severely truncate any expressed protein product. The *dnd1* and *dnd2* mutants are well-suited for use in suppressor screens designed to identify previously unknown genes that influence defense activation and HR cell death. Our Specific Objective is to isolate and study genes that suppress *Arabidopsis dnd1* defense phenotypes. Cloning of suppressor genes will be pursued. Equally important, double-mutant analysis and physiological/molecular biological characterization of suppressed lines will be pursued to place our studies in the broader context of plant defense activation pathways. Methodological tools offered by the *Arabidopsis thaliana* system will be exploited in this project. This work should advance our understanding of plant responses to pathogens, and may also identify targets for manipulation by biotechnologists working to enhance crop plant disease resistance.

2000-02867 A Combinatorial Chemistry Approach to Generate Broad-Spectrum Geminivirus Resistance Genes

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Grant 2001-35319-09887; $135,000; 2 Years

Geminiviruses are a group of plant viruses that cause severe disease problems on a variety of important food and fiber crops throughout the world. In the USA, extensive losses have occurred in tomato and bean crops in Florida and melons in California. Plants are commonly infected with more than one geminivirus, which can make control difficult. These viruses are transmitted by the tropical whitefly and insecticides are extensively used in attempts to control this vector. No widely useful source of natural resistance to these viruses is currently available in the USA, and therefore, the main goal of this project is to provide a strategy that can be used to engineer plants for broad-spectrum resistance to one or more of these viruses. A combinatorial chemistry approach will be used. This approach involves the generation of millions of variants of a small RNA or peptide molecule and the selection of one of these molecules that will inhibit the replication of diverse geminiviruses. A cell tissue culture system will be used to evaluate the effectiveness of these selected molecules to inhibit the replication of *Bean golden yellow mosaic geminivirus* and *Tomato yellow leaf curl geminivirus*. Once these molecules, which inhibit geminivirus replication, are available, then plants can be engineered to express these antiviral molecules, and they should be resistance to diverse geminiviruses

2000-02861 Reproductive Biology of *Russula Brevipes* in Lodgepole Pine Forests

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We propose to study the reproductive biology of an important widespread root-symbiotic fungus, *Russula brevipes,* associated with lodgepole pine. Two genetic markers will be used to document genotypes present in the field, verify the clonality of several subpopulations, determine if each subpopulation was derived from a single mating event, and to directly compare genetic variation in ectomycorrhizae from each subpopulation with the mushrooms that develop there. Long-term genetic variation will be examined in subpopulations of *R. brevipes* by utilizing mushrooms already collected and precisely mapped in the same locations over the past ten years. Sequence analysis will be used in conjunction with an existing database to identify *R. brevipes* and other symbiotic fungi on the roots of lodgepole pine. Laboratory experiments will be conducted on the interaction between basidiospores and mycelia using root chambers that allow growth and nondestructive manipulation and sampling of roots and fungi. This information will allow us to document reproductive history and subpopulation interactions, identify immigration and dispersal events and assess the importance and function of sexual vs. asexual propagules and processes in a symbiotic fungus associated with mature and regenerating forests. Results from

this proposal will provide basic biological information that has been previously unavailable, and is a critical step in understanding how root-symbiotic fungi present in a mature forest make the transition to young forests following disturbance. Such information is required by forest managers before responses to forest fire or clearcutting can be fully understood or adequately predicted.